SN

中华人民共和国出入境检验检疫行业标准

SN/T 4034—2014

进出口化妆品中萘酚的测定
液相色谱-质谱/质谱法

Determination of naphthols in cosmetics for import and export—
LC-MS/MS method

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前 言

本标准按照 GB/T 1.1—2009 给出的规则起草。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国吉林出入境检验检疫局。

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进出口化妆品中萘酚的测定
液相色谱-质谱/质谱法

1 范围

本标准规定了化妆品中 α-萘酚与 β-萘酚的液相色谱-质谱/质谱测定方法。

本标准适用于染发剂、烫发水、弹力素、啫喱水、指甲油、粉底等化妆品中 α-萘酚与 β-萘酚的测定和确证。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文件。凡是不注日期的引用文件，其最新版本（包括所有的修改单）适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

3 方法提要

试样用甲醇溶液提取，经固相萃取柱净化，液相色谱-质谱/质谱仪测定和确证，外标法定量。

4 试剂和材料

除另有规定外，所用试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 甲醇：HPLC 级。

4.2 乙腈：HPLC 级。

4.3 标准物质：α-萘酚（α-naphthol，CAS No.90-15-3，$C_{10}H_8O$），β-萘酚（β-naphthol，CAS No.135-19-3，$C_{10}H_8O$）。纯度均≥98%。

4.4 50%甲醇溶液（体积分数）：取 500 mL 甲醇，用水稀释并定容至 1 000 mL。

4.5 标准储备溶液：1.0 mg/mL。准确称取适量标准品（精确至 0.000 1 g），用甲醇分别配制成浓度为 1.0 mg/mL 的标准储备溶液，0 ℃～4 ℃保存。

4.6 标准工作溶液：从标准储备溶液（4.5）中准确吸取 1.0 mL 于 100 mL 容量瓶中，用甲醇稀释成 10.0 μg/mL 混合标准中间溶液。使用时再根据需要用空白基质液（4.7）稀释配制适当浓度的标准工作溶液，现用现配。

4.7 空白基质液：选择不含萘酚且与待测试样同种类的试样，按照测定步骤 7.1 和 7.2 处理得到空白基质液。

4.8 固相萃取柱（SPE）：聚苯乙烯-二乙烯基苯-吡咯烷酮（PLS），150 mg，6 mL，或相当者；使用前依次用 5 mL 甲醇、5 mL 水活化。

4.9 微孔滤膜：有机系，0.22 μm。

5 仪器与设备

5.1 液相色谱-质谱/质谱仪：配备电喷雾离子源（ESI）。

5.2　超声波发生器。

5.3　天平：感量 0.000 1 g 和 0.01 g。

5.4　离心机：最高转速不低于 10 000 r/min。

5.5　涡旋混合器。

5.6　真空泵。

6　样品制备与保存

取有代表性样品，混匀，于 0 ℃～4 ℃保存。在抽样及制样的操作过程中，应防止样品受到污染或待测物发生含量的变化。

7　测定步骤

7.1　提取

称取 0.5 g 试样（精确至 0.01 g）于 50 mL 离心管中，加入 10 mL 50％甲醇溶液（4.4），涡旋混合 2 min，超声 5 min，以 10 000 r/min 离心 10 min，上清液待净化。

7.2　净化

将 5 mL 待净化液（7.1）过预处理过的固相萃取柱（4.8），待全部通过后，依次以 50％甲醇溶液（4.4）和 5 mL 水洗涤，弃去淋洗液，负压抽干，用 3 mL 甲醇洗脱，收集洗脱液至试管中，40 ℃以下氮气浓缩至近干，用甲醇溶解并定容至 1 mL，经 0.22 μm 滤膜（4.9）过滤，供 LC-MS/MS 测定。

7.3　测定

7.3.1　液相色谱参考条件

液相色谱参考条件如下：

a)　色谱柱：C_{18}，2.1 mm×150 mm，3.5 μm 或相当者；

b)　柱温：室温；

c)　流速：0.25 mL/mim；

d)　进样量：10 μL；

e)　流动相及梯度洗脱条件见表 1。

表 1　流动相及梯度洗脱条件

时间/min	乙腈/%	水/%
0	20	80
6.00	80	20
8.00	80	20
8.01	20	80
20.0	20	80

7.3.2　质谱参考条件

质谱参考条件如下：

a) 电离方式：电喷雾电离（ESI）；
b) 扫描方式：负离子扫描；
c) 检测方式：多反应监测（MRM）；
d) 气帘气（CUR）：30 psi（0.75 MPa）；
e) 雾化气（GS1）：50 psi（1.25 MPa）；
f) 辅助气（GS2）：50 psi（1.25 MPa）；
g) 电喷雾电压（IS）：−4 500 V；
h) 碰撞气（CAD）：6.0 psi（0.15 MPa）；
i) 离子源温度（TEM）：550.0 ℃；
j) 定性离子对、定量离子对、去簇电压、碰撞能量见表 2。

表 2　目标化合物的监测离子对、去簇电压和碰撞能量

化合物名称	母离子 m/z	子离子 m/z	去簇电压 V	碰撞能量 V
α-萘酚	143.0	115.0	−90	−68
		41.2[a]	−90	−68
β-萘酚	143.0	65.0	−90	−35
		115.0[a]	−90	−35
[a] 为定量离子。				

7.3.3　定量测定

根据试样中被测样液中被测组分的含量情况，选取响应值相近的标准工作液进行分析，对于高浓度样品应适当进行系列稀释。标准工作液和样液中被测组分的响应值均应在仪器线性响应范围内。在上述色谱条件下，β-萘酚和 α-萘酚参考保留时间分别约为 9.2 min 和 9.5 min，外标法定量。标准溶液的多反应监测色谱图参见附录 A。

7.3.4　定性测定

标准溶液及样液均按 7.3.1 和 7.3.2 规定的条件进行测定，如果样液中与标准溶液相同的保留时间有峰出现，则对其进行确证。如经确证分析被测组分质量色谱峰保留时间与标准物质相一致，所选择的离子均出现；同时所选择离子的丰度比与标准样物质相关离子的相对丰度一致且在允许偏差之内（见表 3），被确证的样品则可判定为阳性检出。

表 3　定性确证时相对离子丰度的最大允许偏差

相对离子丰度/%	>50	>20～50	>10～20	≤10
允许的相对偏差/%	±20	±25	±30	±50

7.3.5　空白试验

除不称取试样外，均按上述步骤进行。

8 结果计算和表述

按式(1)计算试样中β-萘酚和α-萘酚含量，计算结果应扣除空白值：

$$w_i = \frac{\rho_i \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots(1)$$

式中：

w_i ——试样中被测组分含量，单位为毫克每千克(mg/kg)；

ρ_i ——从标准曲线读出的被测组分溶液浓度，单位为毫克每升(mg/L)；

V ——样液最终定容体积，单位为毫升(mL)；

m ——最终样液代表的试样质量，单位为克(g)。

9 测定低限和回收率

9.1 测定低限

本方法的测定低限均为0.20 mg/kg。

9.2 回收率

不同基质在不同添加水平下的回收率实验数据参见附录B。

附 录 A

（资料性附录）

标准溶液多反应监测（MRM）色谱图[1]

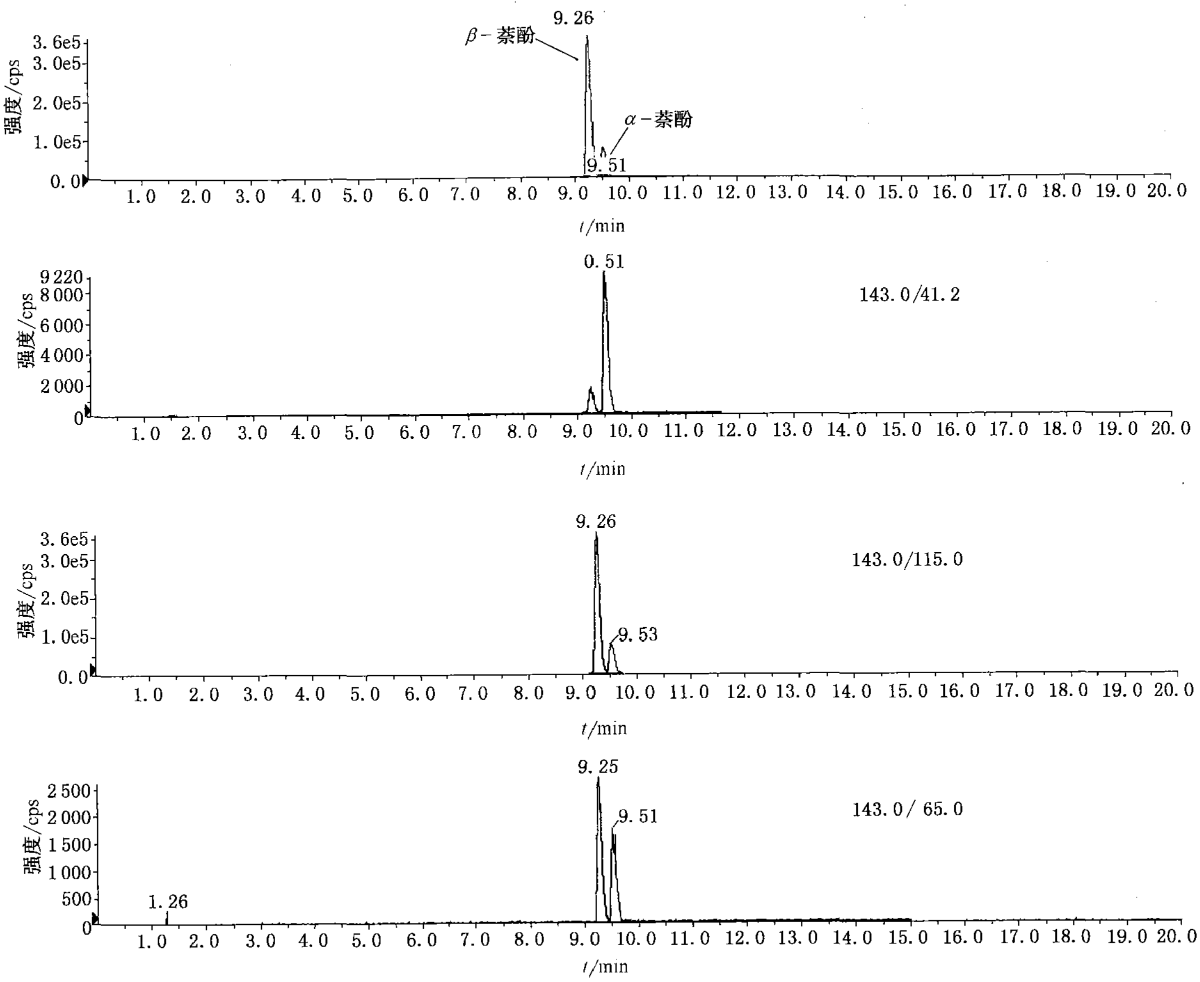

图 A.1 α-萘酚和β-萘酚标准溶液多反应监测（MRM）色谱图及提取离子流图（0.2 mg/L）

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[1] 非商业性声明：附录 A 所列参数是在 API4000 质谱仪上完成的，此处列出试验用仪器型号仅是为了提供参考，并不涉及商业目的，鼓励标准使用者尝试采用不同厂家或型号的仪器。

附 录 B
（资料性附录）
回 收 率

表 B.1 α-萘酚不同添加水平平均回收率（$n=10$）

样品名称	添加浓度	回收率范围/%
弹力素	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	80.0～92.5
	2.00 mg/kg	82.5～93.0
粉底	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	80.0～92.5
	2.00 mg/kg	82.5～90.0
啫喱水	0.20 mg/kg	80.0～105.0
	0.40 mg/kg	80.0～97.5
	2.00 mg/kg	81.0～95.5
染发剂	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	87.5～105.0
	2.00 mg/kg	83.5～93.0
	0.5%	90.0～108.0
烫发水	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	90.0～105.0
	2.00 mg/kg	87.5～97.0
指甲油	0.20 mg/kg	80.0～100.0
	0.40 mg/kg	92.5～107.5
	2.00 mg/kg	83.5～94.5

表 B.2 β-萘酚不同添加水平回收率（$n=10$）

样品名称	添加浓度/(mg/kg)	回收率范围/%
弹力素	0.20	80.0～100.0
	0.40	92.5～107.5
	2.00	83.5～94.5
粉底	0.20	80.0～105.0
	0.40	92.5～105.0
	2.00	93.0～105.5

表 **B.2**（续）

样品名称	添加浓度/(mg/kg)	回收率范围/%
啫喱水	0.20	80.0～95.0
	0.40	90.0～102.5
	2.00	89.5～96.5
染发剂	0.20	85.0～105.0
	0.40	92.5～102.5
	2.00	88.0～96.0
烫发水	0.20	80.0～95.0
	0.40	92.5～107.5
	2.00	90.0～94.5
指甲油	0.20	85.0～105.0
	0.40	95.0～105.0
	2.00	86.5～93.5

Foreword

This standard was drafted in accordance with GB/T 1.1—2009.

Please pay attention that some contents in this standard may refer to patents. The institution doesn't take on the responsibility to identify these patents.

This standard was proposed by and is under the charge of the Certification and Accreditation Administration of the People's Republic of China.

This standard was drafted by Jilin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Kang Mingqin, Li Aijun, Mu Jun, Zhang Daihui, Hu Tingting, Zhao Yunhui.

Determination of naphthols in cosmetics for import and export—LC-MS/MS method

1 Scope

This standard specifies the method for determination of α-naphthol and β-naphthol in cosmetics by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

This standard is applicable for the determination and confirmation of α-naphthol and β-naphthol residues in hair dye、perm water、elastin、gel water、nail enamel and foundation make-up.

2 Cited normative references

The following documents are necessary for this standard. For dated references, only dated editions shall apply to this standard. For undated references, the latest edition of the normative document(including subsequent amendments) referred to applies.

GB/T 6682 Water for analytical laboratory use—Specification and test methods

3 Principle

Liquid sample is extracted with methanol-water, cleaned-up by SPE and determined and confirmed by LC-MS/MS ,quantified by external standard method.

4 Reagents and materials

Unless otherwise specified, all regents used are A. R. and "Water" is grade 1st water specified in GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Acetonitrile: HPLC grade.

4.3 Standard substance (α-naphthol, CAS No. 90-15-3, $C_{10}H_8O$; β-naphthol, CAS No. 135-19-3, $C_{10}H_8O$): Purity≥98%.

4.4 50% Methanol solution(*V*/*V*): Transfer 500 mL into a 1 000 mL beaker, dilute to volume with water an mix well.

4.5 Standard stock solution: 1.0 mg/mL. Accurately weigh appropriate amount of standards(accurate to 0. 000 1 g) and dissolve and dilute to 1.0 mg/mL with methanol. The solution is stored in a frigerator at 0 ℃ ~4 ℃.

4.6 Standard working solution:Dilute the standard stock solution (4.5),with methanol to 10.0 μg/mL as the standard middle solution. According to the requirement, accuately pipet an adequate volume of standard middle solution,dilute with matrix blank solution(4.7) to prepare an appropriate matrix standard working solution.

4.7 Matrix blank solution: The solution of blank matrix handled by step 7.1 and 7.2.

4.8 Solid phase extraction column: Polystyrene-divinyl benzene-pyrrolidone(PLS), 150 mg, 6 mL, or equivalent;pretreat the colume with 5 mL methanol and then with 5 mL water.

4.9 Micro-pore filter film: For organic, 0.22 μm.

5 Apparatus and equipment

5.1 LC-MS/MS: Equipped with electrospray ionization (ESI).

5.2 Ultrasonic generator.

5.3 Balance: 0.000 1 g and 0.01 g sensitivity.

5.4 Centrifuge:The maximum rotate speed is not lower than 10 000 r/min.

5.5 Vortical mixer.

5.6 Vacuum pump.

6 Sample preparation

Take approximate representative sample, shake well and store them at 0 ℃ ~4 ℃. In the course of sampling and sample preparation, it shall be taken to avoid contamination or any factors which may cause the change of residue content.

7 Procedure

7.1 Extraction

Weigh sample of about 0.5 g (accurate to 0.01 g) into a 50 mL centrifuge tube, add 10 mL 50% methanol solution (4.4) and mix thoroughly for 2 minutes.Then extract for 5 min in the ultrasonic processor. Centrifuge for 10 min at 10 000 r/min. The supernatant is to be purified.

7.2 Clean-up

Let 5 mL extrac flow(7. 1)through the SPE column (4.8) which is pretreated. Then wash the column by 5 mL 50% methanol solution(4.4)and 5 mL water, and elute the column by 3 mL methanol which need collection and nitrogen evaporation under 40 ℃. The residue is dissolved with 1.0 mL methanol and mix well by vortical mixer, filtered through 0.22 μm film (4.9) . The filtrate is determined by LC-MS/MS.

7.3 Determination

7.3.1 LC operating condition

LC operating condition referred to next:

a) LC column: ZORBAX SB C_{18}, 2.1 mm × 150 mm, 3.5 μm, or other equivalent;

b) Column temperature: room temp;

c) Flow rate: 0.25 mL/min;

d) Injector volume: 10 μL;

e) Gradient elution conditions see Table 1.

Table 1—Gradient elution conditions

Time/min	acetonitrile/%	water/%
0	20	80
6. 00	80	20
8.00	80	20
8.01	20	80
20.0	20	80

7.3.2 MS/MS operating condition

MS/MS operating condition referred to next:

a) Ionization mode:ESI;

b) Scan mode:negative ion scanning;

c) Detection mode:MRM;

d) Curtain gas (CUR): 30 psi(0.75 MPa);

e) Nebulizer (GS1) :50 psi (1.25 MPa);

f) Auxiliary gas (GS2): 50 psi (1.25 MPa);

g) Ion spray voltage (IS): −4 500 V;

h) Collision gas (CAD): 6.0 psi (0.15 MPa);

i) Probe temperature (TEM) : 550.0 ℃;

j) See Table 2 for quantitative ion, qualitative ion, declustering potential voltage, collision gas energy.

Table 2—Quantitative and qualitative ion pairs, declustering potential voltage and collision gas energy

Compound	parent ion m/z	product ion m/z	Declustering potential voltage V	collision gas energy V
α-naphthol	143. 0	115.0	−90	−68
		41.2[a]	−90	−68
β-naphthol	143.0	65.0	−90	−35
		115.0[a]	−90	−35
[a] represent the quantitative ion.				

7.3.3 LC-MS/MS quantition

According to the approximate concentration of analyte in sample solution, select the standard working solution with similar responses to that of sample solution.The responses of analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Under the above metioned LC-MS/MS operating condition, the retention time of β-naphthol and α-naphthol are 9.2 min and 9.5 min respectively. Reconstituted ion chromatogram of standard working solution is listed in Annex A, quantified by external standard method.

7.3.4 LC-MS/MS confirmation

At the same experimental status(7.3.1 and 7.3.2), when the deviation between the retention time of the test sample and the time of the standard working solution is within ±2.5%, and the allowance between the relative abundance of the qualification ions of the components and the relative abundance of the standard working solution is not over the range described in Table 3, a judgment that the pesticidal residue exists in the sample is confirmed.

Table 3—Maximum permitted tolerances for relative ion intensities while confirmation

Relative ionic abundance/%	>50	>20～50	>10～20	≤10
Allowed relative deviation/%	±20	±25	±30	±50

7.3.5 Blank test

Blank test will be conducted according to the procedures 7.1 and 7.2 without sample addition.

8 Calculation and expression of the result

Calculate the content of α-naphthol and β-naphthol in the test sample according to the followed formula(1),The result of calculation should be deducted with blank value:

$$w_i = \frac{\rho_i \times V \times 1\ 000}{m \times 1\ 000} \quad \cdots\cdots\cdots\cdots (1)$$

Where:

w_i —the residue content of analyte in the test sample,unit is microgram per kilogram (mg/kg);

ρ_i —the concentration of analyte read from the standard working, unit is microgram per liter (mg/L) ;

V —the final volume of the sample solution,unit is milliliter(mL);

m —the corresponding sample weight of the final solution, unit is gram (g).

9 Detection limit and recovery

9.1 Limit of quantification

The limit of quantification of the method is 0.20 mg/kg.

9.2 Recovery

The recoveries of α-naphthol and β-naphthol are in Annex B.

Annex A
(informative)
MRM Chromatogram of standard solution[1]

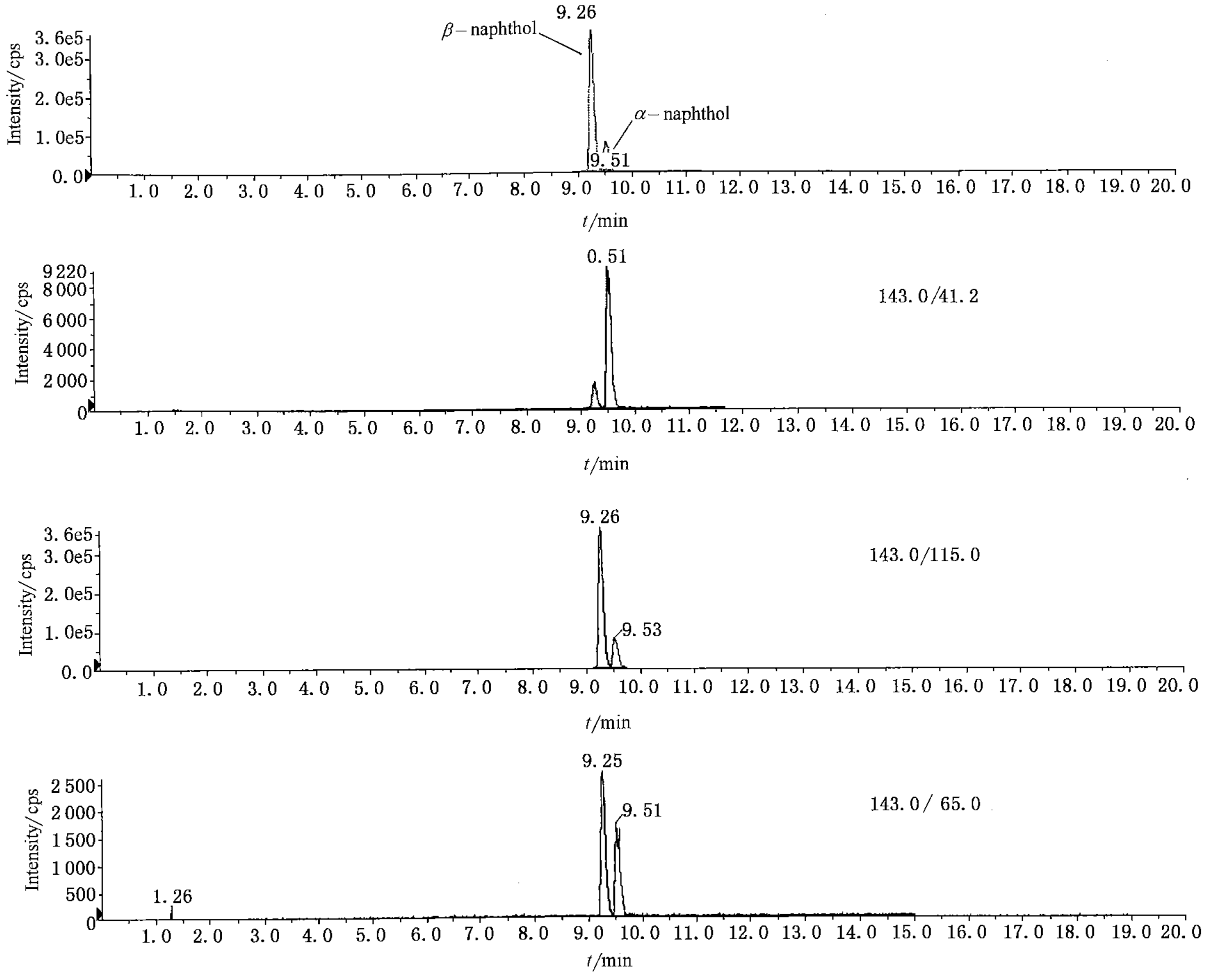

Fig A.1—MRM Chromatogram of α-naphthol and β-naphthol standard solution (0.2 mg/L)

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[1] Non-commercial statement: the reference paramaters in Annex A is accomplished by API4000 LC/MS/MS, the equipment and its model involved in the standard method are only for reference and not related to any commercial aim, and the analysts are encoueaged to use equipments of different corporation or different model.

Annex B
(informative)
Recovery

Table B.1—Recoveries at different levels($n=10$)

Samples	Levals	Recoveries/%
elastin	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	80.0～92.5
	2.00 mg/kg	82.5～93.0
foundation make-up	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	80.0～92.5
	2.00 mg/kg	82.5～90.0
gel water	0.20 mg/kg	80.0～105.0
	0.40 mg/kg	80.0～97.5
	2.00 mg/kg	81.0～95.5
hair dye	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	87.5～105.0
	2.00 mg/kg	83.5～93.0
	0.5%	90.0～108.0
perm water	0.20 mg/kg	80.0～95.0
	0.40 mg/kg	90.0～105.0
	2.00 mg/kg	87.5～97.0
nail enamel	0.20 mg/kg	80.0～100.0
	0.40 mg/kg	92.5～107.5
	2.00 mg/kg	83.5～94.5

Table B.2—β-Recoveries at different levels($n=10$)

Samples	Levals/(mg/kg)	Recoveries/%
elastin	0.20	80.0～100.0
	0.40	92.5～107.5
	2.00	83.5～94.5
foundation make-up	0.20	80.0～105.0
	0.40	92.5～105.0
	2.00	93.0～105.5

Table B.2 (continued)

Samples	Levals/(mg/kg)	Recoveries/%
gel water	0.20	80.0～95.0
	0.40	90.0～102.5
	2.00	89.5～96.5
hair dye	0.20	85.0～105.0
	0.40	92.5～102.5
	2.00	88.0～96.0
perm water	0.20	80.0～95.0
	0.40	92.5～107.5
	2.00	90.0～94.5
nail enamel	0. 20	85.0～105.0
	0.40	95.0～105.0
	2.00	86.5～93.5

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中华人民共和国出入境检验检疫
行　业　标　准
进出口化妆品中萘酚的测定
液相色谱-质谱/质谱法
SN/T 4034—2014
*
中 国 标 准 出 版 社 出 版
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